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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P30925PC01	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/00518	International filing date *(day/month/year)* 16.01.2003	Priority date *(day/month/year)* 17.01.2002
International Patent Classification (IPC) or both national classification and IPC C12N5/08		
Applicant KOBENHAVNS UNIVERSITET et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.08.2003	06.05.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Hoff, C Telephone No. +49 89 2399-7895

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/00518

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-38 as originally filed

Claims, Numbers

1-31 received on 22.04.2004 with letter of 22.04.2004

Drawings, Sheets

1/9-9/9 as originally filed

Sequence listing part of the description, pages:

1-4, filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4, 6-11, 15, 16, 19-30
	No:	Claims	1-3, 5, 12-14, 17, 18, 31
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-31
Industrial applicability (IA)	Yes:	Claims	1-20, 22-24, 29-31
	No:	Claims	

2. Citations and explanations

   see separate sheet

Reference is made to the following documents:

D1: PECHOUX CHRISTINE ET AL: 'Human mammary luminal epithelial cells contain progenitors to myoepithelial cells.' DEVELOPMENTAL BIOLOGY, vol. 206, no. 1, 1 February 1999 (1999-02-01), pages 88-99, XP002216780 ISSN: 0012-1606 cited in the application

D2: SMALLEY MATTHEW J ET AL: 'Differentiation of separated mouse mammary luminal epithelial and myoepithelial cells cultured on EHS matrix analyzed by indirect immunofluorescence of cytoskeletal antigens.' JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY, vol. 47, no. 12, December 1999 (1999-12), pages 1513-1524, XP002216788 ISSN: 0022-1554 cited in the application

The IPER has been drafted assuming that the application is entitled to the priority of 17.01.02.

V.1 Novelty

V.1.2 The subject matter of claims 1-3, 5, 12-14, 17,18, 31 is not novel according to Article 33(2) PCT.

D1 discloses the isolation of human mammary luminal epithelial cells able to convert into myoepithelial cells ((p.94, right col, 2nd paragraph). As a consequence the subject matter of claims 1-3, 5, 17, 18, 31 is not novel. Said cell express the marker CK19 (p.94, left col, 2nd paragraph). Therefore D1 also destroys the novelty of claim 12.

D2 discloses the isolation of mouse mammary luminal epithelial cells able to convert into myoepithelial cells and luminal epithelial cells (p.1522, right col, 3rd paragraph). Said cells express the marker CK19. As a consequence the subject matter of claims 1, 2, 5, 12-14, 17, 18, 31 is not novel.

D1 and D2 both disclose isolated cells from luminal epithelial cells of a mammary gland. Said cells cannot be differentiated from the cells disclosed in claim 1 as already mentioned above. The cells of D1 and D2 were not shown to be positive for the luminal

marker ESA and negative for sialomucin but since the expression of these marker was not tested the cells are considered as inherently presenting the same marker expression. Accordingly claim 1 is not considered as fulfilling the requirements of novelty

V.1.2 The subject matter of claims 4, 6-11, 15, 16, 19-30 is considered as fulfilling the requirements of Article 33(2) PCT.

V.2 Inventive step

The subject matter of claims 4, 6-11, 15, 16, 19-30 is not considered as involving an inventive step according to Article 33(3) PCT.

The subject matter of claims 4, 6-11, 15, 16 refers to the immortalisation of cell lines. Such immortalisation processes involve routine methods which are known from the prior art. Therefore claims 4, 6-11 are not considered as inventive according to Article 33(3) PCT.

The subject matter of claims 19-26 involves routine methods. Such methods can be applied to any cell line or were already applied to known cell lines. Consequently the patentability of said methods depends on the patentability of the main claims. Since the main claims are at present not patentable under Article 33(3) said claims are also not considered as patentable. The same applies for the subject matter of claims 29 and 30.

The subject matter of claims 27 and 28 is entirely speculative. As a consequence said claims are, at present, not considered as fulfilling the requirements of Article 33(3) PCT.

V.3 Additional comments

V.3.1 For the assessment of the present claims 21, 25-28 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

V.3.2 Claim 1

As presently formulated, claim 1 does not relate to an isolated cell derived from luminal epithelial cells of a mammary gland which is ESA+ and MUC-. Instead it refers to isolated cells derived from luminal epithelial cells of a mammary gland capable of forming a cell culture comprising cells which are ESA+ and MUC-. From claim 1 it is clear that the cells are **cultured between the isolation and the analysis of the marker expression**. Indeed the cells which are found to be ESA+ and MUC- are taken from the cell culture meaning that, depending on how long and under which conditions the cells are maintained in culture, the differentiation of the cell is in process or already done.

Claims

1. An isolated cell, derived from luminal epithelial cells of a mammary gland, which is capable of proliferating and capable of differentiating into cells of mammary gland luminal epithelial and myoepithelial cell lineages.

2. A cell according to claim 1 which is isolated from suprabasal luminal epithelial cells of the mammary gland.

3. A cell according to claim 2 which is a human cell.

4. A cell according to any of claims 1 - 3 which is capable of forming a cell culture comprising cells which are positive staining for the luminal epithelial marker ESA (ESA+) and negative or weakly positive staining for sialomucin (MUC-), (ESA+/MUC-) cells.

5. A cell according to any of claims 1 - 4 which is immortalised.

6. A cell population composed of cells according to any of claims 1 - 5.

7. An immortalised cell line derived from the cell of claim 5.

8. An immortalised cell line according to claim 7, wherein the immortalising step comprises transfecting the cells with a nucleic acid molecule encoding an immortalising polypeptide.

9. An immortalised cell line according to claim 8, wherein the immortalising step comprises transfecting the cells with a nucleic acid molecule encoding a papillomavirus polypeptide selected from the group consisting of E6, E7 and a nucleic acid molecule comprising E6 and E7.

10. An immortalised cell line according to claim 8, wherein the immortalising step comprises transforming the cells with at least one retroviral vector including an expression cassette comprising a nucleic acid molecule encoding a papillomavirus polypeptide selected from the group consisting of E6, E7 and a nucleic acid molecule comprising E6 and E7, and selecting the immortalised cells.

11. An immortalised cell line according to claim 10, wherein the immortalising step is performed by transforming the cells with retrovirus-containing supernatant from the PA317 LXSN HPV16E6E7 cell line and selecting the immortalised cells.

12. An immortalised cell line according to any of claims 7 - 11 that in culture is capable of forming branching structures resembling terminal duct lobular units of the mammary gland in morphology and/or by marker expression.

13. An immortalised cell line according to any of claims 7 - 12 which comprises cells that are positive staining for the keratin K19.

14. An immortalised cell line according to any of claims 7 - 13 that is derived from a cell selected from the group consisting of a rodent cell, a porcine cell, a ruminant cell, a bovine cell, a caprine cell, a equine cell, a canine cell, a ovine cell, a feline cell and a primate cell.

15. An immortalised cell line according to claim 14 that is selected from the group consisting of cells from mice, rats and rabbits.

16. An immortalised cell line according to claims 14 that is a human cell line.

17. The immortalised cell line according to claim 7 which is deposited in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and has obtained the accession number DSM ACC 2529.

18. A method for isolation of an at least bi-potent mammary gland tissue cell, comprising the steps of:

(i) separating said tissue into two or more different cell types

(ii) culturing each of said different cell types under cell differentiation conditions and

(iii) selecting the cell type(s) that is/are capable of differentiating into at least two morphologically and/or phenotypically different cell types.

19. A method according to claim 18 in which the at least bi-potent cell is a cell according to any of claim 1-5.

20. A method for testing the toxic effect, if any, of a substance on mammary gland epithelial cells, the method comprising:

(i) culturing or maintaining the cells of any of claims 1 - 17 in a non-toxic medium;

(ii) adding the substance to be tested to the medium; and

(iii) determining the response, if any, of the cells, including changes in cell growth rate, cell death rate, apoptosis, cell metabolism, inter- as well as intra-cellular communication, morphology, mRNA or protein expression and antigen expression.

21. A method for testing the carcinogenic effect, if any, of a substance on mammary gland epithelial cells, the method comprising:

(i) culturing the cells of any of claims 1 - 17 in a growth medium which maintains the cells as non-transformed cells;

(ii) adding the agent, compound or factor under test to the cell culture; and

(iii) determining the neoplastic response, if any, of the so contacted cells by changes in morphology, tumorigenicity in animals, mRNA expression and/or antigen expression as well as other changes which is associated with carcinogenicy.

22. A method as claimed in claim 21, wherein the tumorigenicity test comprise the introduction af said treated cells into an immune incompetent test animal.

23. A method of testing the ability, if any, of a substance to modulate the differentiation of non-terminal differentiated mammary gland epithelial cells, the method comprising:

(i) culturing or maintaining the cells of any of claims 1 - 17 in a medium which in tiself does not modulate the differentiation;

(ii) adding the substance under test to the cell culture; and

(iii) determining the differentiation modulation responses, if any, of the so contacted cells by changes in cell growth rate, cell death rate, apoptosis, cell metabolism, inter- as well as intra-cellular communication, morphology, mRNA or protein expression or antigen expression as well as other changes which is associated with differentiation.

24. A method for screening a substance for its ability, if any, to interact with a cellular protein, the method comprising:

(i) transfecting a cell of any of claims 1 - 17 with a gene construct enabling transfected cells to express said protein;

(ii) adding the substance to be tested to the cells; and

(iii) determining the interaction, if any, with a cellular protein by changes in cell growth rate, cell death rate, apoptosis, cell metabolism, inter- as well as intra-cellular communication, morphology, mRNA or protein expression, antigen expression or other changes which either directly or indirectly is supposed to be associated with said protein.

25. A method according to claim 24 in which said cellular protein is selected from the group consisting of estrogen receptor-alpha, estrogen receptor-beta and progesterone receptor.

26. A method of transplanting a vertebrate host with a cell according to any of claims 1-5, comprising the step of introducing the cell into the vertebrate host.

27. A method of *in vivo* administration of a protein or gene of interest to an individual in need thereof, comprising the step of transfecting the cell-population of any of claims 1 - 5 with a vector comprising DNA or RNA which expresses the protein or gene of interest and introducing the transfected cell into said individual.

28. Use a cell according to any of claims 1 - 5 to prevent and/or treat cellular debilitations, derangements and/or dysfunctions and/or other disease states in mammals, comprising administering to a mammal a therapeutically effective amount of said cells, or cells or tissues derived therefrom.

29. A method of tissue repair or transplantation in mammals, comprising administering to a mammal a therapeutically effective amount of a cell according to any of claim 1 - 5, or cells or tissues derived therefrom.

30. A pharmaceutical composition comprising: a therapeutically effective amount of a cell according to any of claims 1 - 5, or cells or tissues derived therefrom; and a pharmaceutically acceptable carrier.

31. The pharmaceutical composition of Claim 30 further comprising a proliferation factor or lineage commitment factor.

32. A diagnostic agent comprising the cell of any of claim 1 - 5, or any part thereof.